IN THE DRAWINGS

Please replace Fig. 7 with the replacement Fig. 7 submitted herewith. The replacement sheet is needed to correct a typographical error in the legend as requested by the Examiner. A marked-up copy of the original sheet to show the change is also submitted herewith.

REMARKS

The Official Action of 3 May 2006 has been carefully considered and reconsideration of the application as amended is respectfully requested.

The specification has been amended to make reference to primers 5, 8, 61 and 71 of the drawings and a replacement Fig. 7 has been submitted to remove the bases for the Examiner's objections at paragraph 1 of the Official Action. Applicants respectfully note that, with respect to primers 1, 4, 21 and 31, reference is already made in the specification at page 8, line 29.

The specification has also been amended to correct the informalities courteously noted by the Examiner at paragraph 2 of the Official Action. The embedded hyperlink on page 16 has been removed as requested by the Examiner on page 3 of the Official Action.

Claim 1 has been amended more clearly to distinguish over the cited art by the incorporation of the "consisting of" transitional to limit the primers in the recited multi-cyclic polymerase chain reactions to the primers as recited in the claim. Claim 1 has also been amended to remove the basis for the objection appearing at page 4 of the Official Action. Other claims have been amended to render them more definite without narrowing the scope thereof. New claims 16-18 have been added more completely to define the subject matter which Applicants regard as their invention. Support for the recitations in claim 18

appears in the Sequence Listing in the specification, wherein it is shown that the described primers comprise 36 or fewer nucleotide sequences.

Claims 1-15 are rejected under 35 USC 112, second paragraph, as allegedly being indefinite. Applicants respectfully traverse the rejection, and respectfully submit that the Examiner is confusing the terms "template" and "target polynucleotide sequence".

Regarding "template" as used in step (2) of Claim 1, it is the product obtained in the previous polymerase chain reaction and contains only a fragment of the "target polynucleotide sequence" just synthesized in the previous polymerase chain reaction and the starting material, i.e., "first template." A fragment of the "target polynucleotide sequence" to be synthesized in a polymerase chain reaction may be **absent** in the "template." Please also see page 7, lines 22 to 26 of the specification: "[T]he method is initiated with a first template that is any template sequence commonly used in the host-vector expression system or a fragment of the target polynucleotide. The present invention is characterized in that a template that is highly relevant to the target polynucleotide is not necessary."

Regarding the "target polynucleotide sequence," please see page 8, lines 6 to 10, which recites that "[A]s used herein, the term "target polynucleotide sequence" refers to a sequence to be produced. The polynucleotide molecule corresponding to the target polynucleotide sequence may not be available or

even may not exist in nature. On the other hand, the target polynucleotide sequence may be a sequence coding for a protein or a peptide."

The fragment of the "target polynucleotide sequence" in the primer is **not** added into the template before the claimed polymerase chain reaction takes place. Taking (i) primers in step (2) of Claim 1 as an example (also see the right column of FIG. 1), the part (a1) of the forward primer located at the 5'-end region of the forward primer does **not** allow hybridization to the 3'-end of target sequence. To the contrary, it is **homologous** to the fragment at the 3'-end region of the target polynucleotide sequence. The part (b1) located at the 3'-end region of the forward primer is homologous to the sequence of the 5'-end region of the template sequence and allows hybridization to the 3'-end of the complementary strand of the template. Therefore, the 5'-end of the forward primer is unanchored since the fragment of the "target polynucleotide sequence" to be synthesized is still absent. On the other hand, the 3'-end of the forward primer is anchored to the complementary strand of the template sequence, and the forward primer along with the reverse primer is able to carry on a DNA synthesis. Similarly, the parts (a3), (b3), (c3) and (d3) of the primer (iii) and the parts (a2) and (b2) of the primer (ii) in step (2) of Claim 1 are able to carry on a polymerase chain reaction.

Claims 1-4 and 10-11 stand rejected under 35 USC 102(b) as allegedly being anticipated by Horton et al. Claims 5-6 stand rejected under 35 USC 103(a) as allegedly being unpatentable over Horton et al in view of Jayaraman et al as evidenced by Springer and Sligar. Claims 7-9 and 12-15 stand rejected

under 35 USC 103(a) as allegedly being unpatentable over the aforementioned combination of references and further in view of Baneyx. Applicants respectfully traverse these rejections.

Horton et al describe a method for **splicing first and second genes** , wherein each of the strands of two (2) genes is amplified in a PCR reaction. Each strand of the first gene is amplified with primers a and b in a first reaction, and each strand of the second gene is amplified with primers c and d in a second reaction. Primers b and c are selected such that the first and second reactions generate two (2) PCR products with homologous segments aligned (see Horton et al at page 63, Fig. 1). In a third reaction, the PCR products are mixed along with excess primers a and d. As noted in the legend of Horton's Fig. 1, the ends of the PCR products with the homologous segments act as primers for one another. Thus, as shown in the hatched box in Fig. 1, the third reaction proceeds with **three (3) primers**: a, d and one of the homologous PCR products (assuming that the other homologous PCR product is considered as a "template"), and the third reaction acts on **three (3) templates** to provide **three (3) separate reaction products**: fragment AB, fragment CD and the Recombinant Product (see Horton et al at Fig. 1). Two (2) of these three (3) reaction products are the same length as the previous reaction product.

In contrast, the claimed invention is a method for **synthesizing a target polynucleotide**, wherein the multi-cyclic PCR reactions are conducted with primers that are designed for extending and amplifying a single template

sequence only. In the claimed method, it is not desirable to amplify both strands of a second gene for splicing of the second gene with the template gene fragment, and the primers thus consist only of a forward primer and a reversed primer which are used in PCR to form a single reaction product. In line with this mechanism, the "consisting of" recitations of the claims as amended exclude from the multi-cyclic polymerase chain reactions additional primers, such as those required n Horton et al, which do not amplify and extend the template sequence (see MPEP 2111.03). The recitation that the multi-cyclic PCR is conducted only on a template that **consists of** a product that has been extended in a previous polymerase chain reaction whereby each succeeding PCR product is longer than the PCR product of each previous polymerase chain reaction (see claim 19) is not met by the Horton et al method, which as discussed above uses templates for PCR that result in products of the same length.

Applicants respectfully note that, in the absence of the hindsight provided by the present specification, there could have been no motivation to modify Horton et al to arrive at the claimed method. As noted above, Horton et al's method is designed to splice first and second (double stranded) genes, and necessitates the amplification of each strand of both genes using the primers described in the reference. In these circumstances, a modification of the Horton et al method would impermissibly change the principle of operation of the reference. See MPEP 2143.01(VI) ("If the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render

the claims *prima facie* obvious."). Accordingly, there is nothing in Horton et al or in any of the secondary references that would have motivated one of skill in the art to modify Horton et al to arrive at the claimed method.

With specific respect to the subject matter of claim 18, the method is additionally patentable over the cited art for another reason. The process in the Horton reference uses primers synthesized in a polymerase chain reaction, and the overlap between the primers and template is applied in the primer extension. Because the primers in the Horton reference are synthesized in a polymerase chain reaction, a template that is homologous to the target sequence must be obtained. For example, the templates, H-2K^{b} and H-2L^{d}, must be provided for synthesizing the primers of AB, CD, EF or GH. In contrast, the primers according to the claimed invention contain no more than 36 nt (ORF7-C-R0, SEQ ID NO: 3) and may be chemically synthesized. A template that is homologous to the target polynucleotide sequence is not necessary. As described on page 8, lines 6 to 10: "[A]s used herein, the term "target polynucleotide sequence" refers to a sequence to be produced. The polynucleotide molecule corresponding to the target polynucleotide sequence may not be available or even may not exist in nature." The primer sequence can be synthesized at will. Furthermore, a polymerase chain reaction using long primers (as described in the Horton reference) is not easily performed, and specific conditions are usually required. In contrast, the polymerase chain reaction using short primers according to the claimed invention is easily controlled, and routine conditions are applicable.

With specific respect to the subject matter of claim 2, the Horton reference illustrates at page 66, paragraph 2 and Fig. 4 a primer recovery step. The fragments of AB, CD, EF and GH in the first templates of H-2K^d and H-2L^d still exist in the final product. In contrast, in the claimed method the first template is removed from the final product. As discussed in the present specification: "[A]ccording to the invention, the method further comprises a step of removing the nucleotide sequence of the first template from the final product in the step (3) so as to obtain the target polynucleotide sequence if the first template is irrelevant to the target polynucleotide sequence, such as the template sequence commonly used in the host-vector expression system." See page 14, lines 15 to 19. In the optional step according to FIGs. 1 and 2, the first template is totally removed from the final product.

With specific respect to the subject matter of Claim 10, the mutant sites produced in the Horton reference are much fewer than those that can be produced according to the claimed invention. Because the primers in the Horton reference are synthesized in a polymerase chain reaction, only the sites of the primers a, b, c, d, e, f, g, or h can be mutated, and the sites between the primers cannot be mutated. In contrast, every site can be mutated with the claimed method.

With specific respect to the subject matter of claims 5 and 9, the claimed method comprises a step of adjusting a sequence of one of **the primers** to change a codon to one that will make expression of the target sequence more efficient. The method for changing codons as taught in the Jayaraman reference differs from that

claimed in the. Referring to page 4085, column 1, paragraph 2 of the Jayaraman reference, the method comprises a polymerase chain reaction, recovery of polymerase chain reaction product, and ligation. In contrast, the recovery and ligation steps are unnecessary in the method according to the claimed invention. In addition, the François and Springer references both fail to disclose the polymerase chain reaction and are thus incompetent to supplement the deficiencies in the oher references.

In view of the above, Applicants respectfully submit that all rejections and objections of record have been overcome and that the application is now in allowable form. An early notice of allowance is earnestly solicited and is believed to be fully warranted.

Respectfully submitted,

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